Supplementary Information

The dynamin-related protein DRP-1 and the insulin signaling pathway cooperate to modulate *C. elegans* longevity

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Supplementary Materials and Methods:

Strains:

The strains used were N2 (wild-type), *age-1(hx546)*, *daf-2(e1370)* and *drp-1(tm1108)*. The *drp-1(tm1108)* strain, which has no detectable DRP-1 protein expression, is likely to represent a strong loss-of-function or null mutant (Breckenridge *et al*. 2008). The *age-1(hx546);drp-1(tm1108)* and the *daf-2(e1370);drp-1(tm1108)* strains were constructed using standard genetic methods, and at least 2 independent isolates of each strain were tested for lifespan and stress resistance. All strains were out-crossed into wild-type N2 in our lab at least 4 times and cultured using standard methods (Brenner 1974).

Generation of the drp-1 transgene under control of endogenous drp-1 promoter:

A 3.7Kbp genomic fragment containing the 0.6Kbp sequence upstream of the *drp-1* coding region, the 2.8Kbp *drp-1* coding region and the 0.3Kbp sequence downstream of the *drp-1* coding region were amplified from genomic DNA by PCR. The construct was verified by sequencing and restriction fragment length analysis. Sequences of the primers used for PCR are available upon request.

Generation of the drp-1 transgenic animals:

10ng/ul of the *drp-1* transgene and 50ng/μl of P*mec-7::rfp* as a co-injection marker and 50ng/μl Bluescript plasmid filler DNA were micro-injected into the gonads of adult hermaphrodite wild-type animals using standard methods (Berkowitz *et al*. 2008). F1 progeny were selected on the basis of RFP fluorescence. Individual F2 worms were isolated to establish independent lines. The worms used as controls for the lifespan and heat stress experiments were injected with 50ng/μl of P*mec-7::rfp* as a co-injection marker and 60ng/μl Bluescript plasmid filler DNA.

Lifespan assays:

Lifespan assays were performed as in (Li *et al*. 2008) with slight modifications. The worms were allowed to lay eggs at 20°C for 2-4 hours. All lifespan assays were performed at 20°C with a 48 hour shift to 25°C when the worms reached adulthood (to avoid worm bursting). Worms were transferred onto fresh plates every other day until Day 14 of adulthood and then every 4-6 days until the end of the assay. Each lifespan assay was performed on triplicate plates in at least 2 independent experiments.

DAF-16 sub-cellular localization:

daf-2(e1370);daf-16(mgDf47);xrls87 transgenic worms were allowed to lay 30 eggs on plates seeded with RNAi bacteria containing the empty vector L4440 or *drp-1* RNAi. DAF-16 sub-cellular localization was scored every day based on a four-category system as in (Padmanabhan *et al*. 2009) using a Leica MZFLIII microscope. The progeny were grown at 16°C until they reached the gravid adult stage (Day 0), placed at 25°C (the permissive temperature for the *daf-2(e1370)* strain) from Day 0 to Day 2 and then kept at 20°C for the remainder of the experiment. DAF-16::GFP nuclear localization was responsive to temperature shift (from 16°C to 25°C at Day 0) but not to *drp-1* RNAi (data not shown). The data obtained at Day 3 adulthood are

presented in Fig.2D. Worms were transferred into freshly induced plates at Days 0, 2 and 4. Each assay was performed on triplicate plates for each strain in 3 independent experiments.

Mitochondrial network visualization upon drp-1 mutation:

Gravid adults were allowed to lay 30 eggs on NGM plates seeded with OP50 and 0.25µM tetramethylrhodamine (TMRE). The progeny were kept at 16°C until they reached the L4 stage. Confocal images of the tail area of live L4 worms immobilized in 3.3mM levamisole on a 2% agarose pad were taken on a Leica TCS SP2 using a 63X water objective and electronic zoom to focus on the mitochondrial network near the top surface of the worm. *ImageJ* was used to obtain values for area, perimeter, major and minor axis lengths for an estimated ellipse, and circularity (calculated by 4*pi*Area/Perimeter^2) for each mitochondrion in an image, ignoring mitochondria <5 square-pixels or on the edge of the image. There were typically ~80-200 mitochondria per image, and the values for each mitochondrion were first averaged for each image (the data presented are from at least 15 images by strain).

Mitochondrial network visualization upon drp-1 RNAi:

Mitochondria in the body wall of the worms were visualized using a *Pmyo-3:mito::GFP* construct in L4 larvae of wild-type worms fed with RNAi bacteria carrying L4440 empty vector or *drp-1* RNAi clone. Images were taken on a Leica DM 5000B.

Supplementary Figure Legends:

Supplementary Figure 1: *drp-1* RNAi affects mitochondrial morphology in muscle cells of the body wall. GFP expression of a *Pmyo-3::GFPmt* transgene in wild-type worms treated with empty vector in **A** or *drp-1* RNAi in **B**.

Supplementary Figure 2: The extended longevity and the sensitivity to heat stress of the *age-1;drp-1* mutant compared to the *age-1* mutant can be rescued by introduction of a transgene overexpressing *drp-1*.

(A) *age-1;drp-1* and 2 independent lines of *age-1;drp-1;rwEx15[Pmec-7::RFP]* mutant worms lived longer than *age-1* and 2 independent lines of *age-1;drp-1;rwEx15[drp-1+Pmec-7::RFP]* mutant.

(B) *drp-1*, *age-1;drp-1* and *age-1;drp-1;rwEx15[Pmec-7::RFP]* mutant worms are more sensitive to heat stress than wild-type, *age-1* and 2 independent lines of *age-1;drp-1;rwEx15[drp-1+Pmec-7::RFP]* mutant worms.

Supplementary Tables

Supplementary Table 1: Quantitative data and statistical analyses of mean adult lifespan presented in Figure 1.

Strain	Mean adulthood lifespan +/- s.d (days)	Censored worms (%)	n	Mean adulthood lifespan +/- s.d (days)	Censored worms (%)	n	p-value (stratified log-rank test) compared to control RNAi	Number of experiments pooled
	Control RNAi			***drp-1*** RNAi				
Wild-type	19.21 +/- 0.14	13.8	586	19.27 +/- 0.13	4.2	672	0.430	9
age-1(hx546)	26.89 +/- 0.46	23.6	505	40.72 +/- 0.36	7.4	514	<0.001	7
daf-2(e1370)	34.67 +/- 1.24	6.7	180	51.02 +/- 1.20	5.6	233	<0.001	3
	daf-16 RNAi			**p-value (stratified log-rank test) compared to single mutant counterpart**			**Number of experiments pooled**	
Wild-type	12.65 +/- 0.07	0.0	301				2	
drp-1(tm1108)	12.10 +/- 0.10	0.0	247	<0.001			2	
age-1(hx546)	13.24 +/- 0.11	6.5	275				2	
age-1 (hx546);drp-1(tm1108)	12.86 +/- 0.14	0.5	222	0.079			2	
daf-2(e1370)	16.05 +/- 0.18	11.1	296				2	

daf-2 (e1370);drp-1(tm1108)	16.34 +/- 0.20	0.0	183	0.889	2

Strain	**Mean adulthood lifespan +/- s.d (days)**	**Censored worms (%)**	**n**	**p-value (stratified log-rank test)**
Wild-type	15.15 +/- 0.10	2.1	1007	
drp-1(tm1108)	15.44 +/- 0.08	2.5	922	0.131 *compared to wild-type worms*
age-1(hx546)	26.87 +/- 0.31	5.9	624	
age-1 (hx546);drp-1(tm1108) isolate A	37.76 +/- 0.40	1.6	565	<0.001 *compared to age-1 mutant*
age-1 (hx546);drp-1(tm1108) isolate B	33.49 +/- 0.36	5.5	495	<0.001 *compared to age-1 mutant*
daf-2(e1370)	28.57 +/- 0.83	15.2	348	
daf-2 (e1370);drp-1(tm1108)	49.47 +/- 1.14	9.7	318	<0.001 *compared to daf-2 mutant*

Supplementary Table 2: Effect of *drp-1* RNAi on the mean lifespan of long-lived worms undergoing bacteria deprivation and long-lived mitochondrial mutant worms.

Strain	Mean adulthood lifespan +/- s.d (days)	Censored worms (%)	n	Mean adulthood lifespan +/- s.d (days)	Censored worms (%)	n	p-value (stratified log-rank test) compared to control RNAi	Number of experiments pooled
	Control RNAi			***drp-1*** RNAi				
Wild-type fed *ad-libitum*	15.03 +/- 0.13	3.8	65	15.54 +/- 0.24	4.7	70	0.257	1
Wild-type food-deprived	35.43 +/- 1.40	15.2	59	38.23 +/- 1.35	12.8	45	0.134	1
isp-1(qm150); ctb-1(qm189)	24.55 +/- 0.34	14.1	3 1 1	26.90 +/- 0.38	10.0	299	<0.001	4
clk-1(e2519)	24.60 +/- 0.35	13.9	1 1 5	26.54 +/- 0.34	3.5	141	<0.001	2

Supplementary Table 3: Quantitative data and statistical analyses of survival on paraquat presented in Figure 2.

Strain	Mean adulthood lifespan +/- s.d (days)	Censored worms (%)	n	p-value (stratified log-rank test) compared to wild-type	p-value (stratified log-ranktest) compared to *age-1(hx546)*	Number of experiments pooled
Wild-type	3.07 +/- 0.04	14.3	407		<0.001	4
drp-1(tm1108)	2.81 +/- 0.03	25.0	344	<0.001	<0.001	4
age-1(hx546)	4.34 +/- 0.05	16.1	616	<0.001		4
age-1 (hx546);drp-1(tm1108) isolate A	3.861 +/- 0.12	22.5	187	<0.001	<0.001	4
age-1 (hx546);drp-1(tm1108) isolate B	3.52 +/- 0.05	14.0	385	<0.001	<0.001	4

References

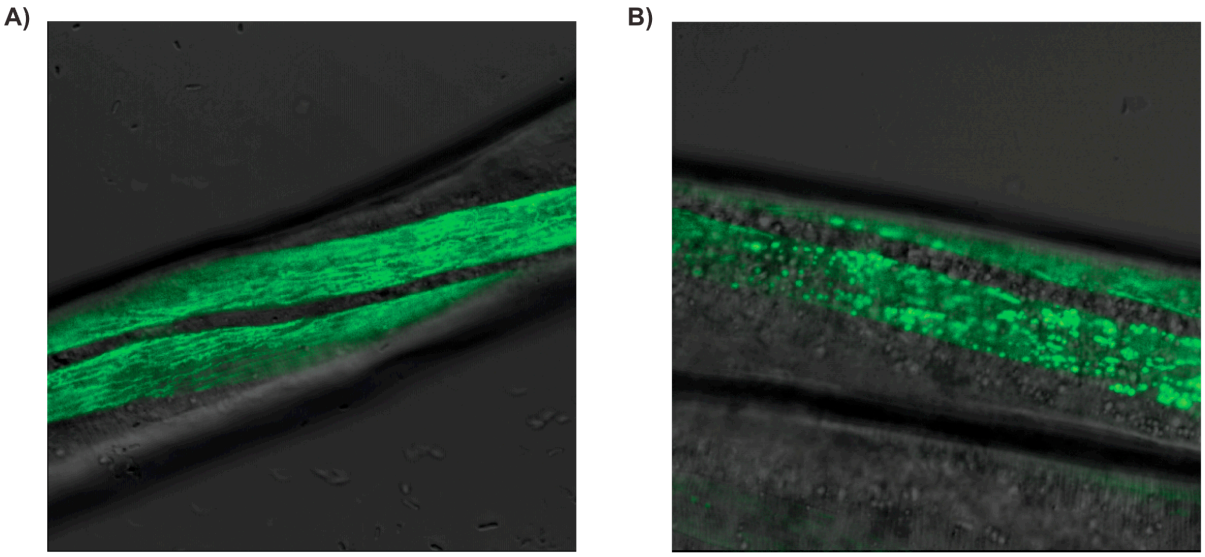
Berkowitz LA, Knight AL, Caldwell GA , Caldwell KA (2008). Generation of stable transgenic C. elegans using microinjection. *J Vis Exp*.

Breckenridge DG, Kang BH, Kokel D, Mitani S, Staehelin LA , Xue D (2008). Caenorhabditis elegans drp-1 and fis-2 regulate distinct cell-death execution pathways downstream of ced-3 and independent of ced-9. *Mol Cell*. **31**, 586-597.

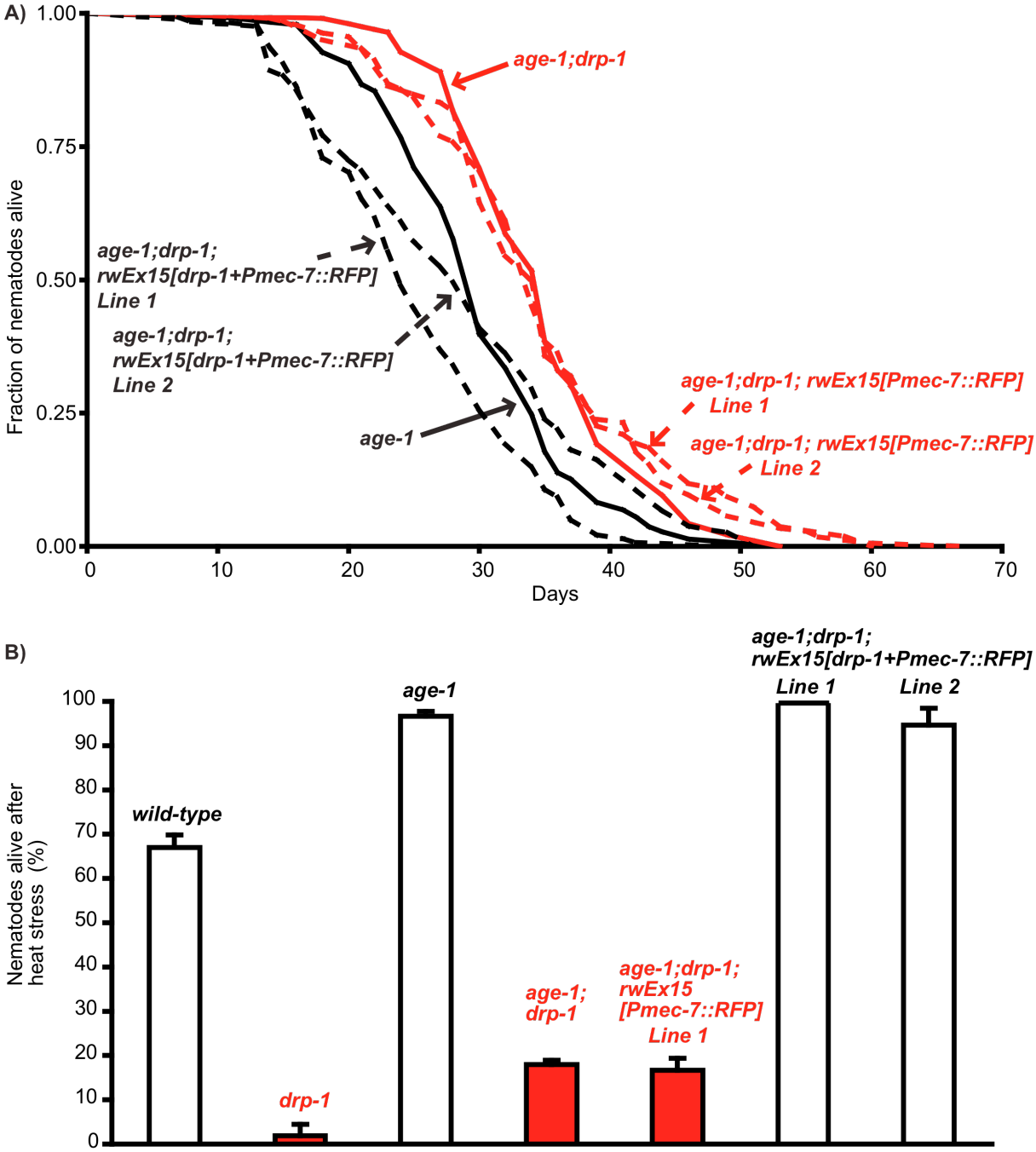
Brenner S (1974). The genetics of Caenorhabditis elegans. *Genetics*. **77**, 71-94.

Li J, Ebata A, Dong Y, Rizki G, Iwata T , Lee SS (2008). Caenorhabditis elegans HCF-1 functions in longevity maintenance as a DAF-16 regulator. *PLoS Biol*. **6**, e233.

Padmanabhan S, Mukhopadhyay A, Narasimhan SD, Tesz G, Czech MP , Tissenbaum HA (2009). A PP2A regulatory subunit regulates C. elegans insulin/IGF-1 signaling by modulating AKT-1 phosphorylation. *Cell*. **136**, 939-951.



Supplementary Figure 1: *drp-1* RNAi affects mitochondrial morphology in the muscle cells of the body wall. GFP expression of a *myo-3-GFPmt* transgene in wild-type worms treated with empty vector **(A)** or *drp-1* RNAi **(B)**.



Supplementary Figure 2: The extended longevity and the sensitivity to heat stress of the *age-1;drp-1* mutant compared to the *age-1* mutant can be rescued by introduction of a transgene overexpressing *drp-1*.
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(B) *drp-1*, *age-1;drp-1* and *age-1;drp-1;rwEx15[Pmec-7::RFP]* mutant worms were more sensitive to heat stress than wild-type, *age-1* and 2 independent lines of *age-1;drp-1;rwEx15[drp-1+Pmec-7::RFP]* mutant worms.